Abnormal sarcoplasmic reticulum Ca\textsuperscript{2+}-sequestering properties in skeletal muscle in chronic obstructive pulmonary disease


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Green HJ, Burnett M, Duhamel TA, D’Arsigny C, O’Donnell DE, Webb KA, Ouyang J. Abnormal sarcoplasmic reticulum Ca\textsuperscript{2+}-sequestering properties in skeletal muscle in chronic obstructive pulmonary disease. Am J Physiol Cell Physiol 295: C350–C357, 2008. First published May 28, 2008; doi:10.1152/ajpcell.00224.2008.—The objective of this study was to investigate the hypothesis that alterations in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+}-cycling properties would occur in skeletal muscle in patients with moderate to severe chronic obstructive pulmonary disease (COPD). To investigate this hypothesis, tissue samples were obtained from the vastus lateralis of 8 patients with COPD [age 65.6 ± 3.2 yr; forced expiratory volume in 1 s (FEV\textsubscript{1})/forced vital capacity (FVC) = 44 ± 2%; mean ± SE] and 10 healthy age-matched controls (CON, age 67.5 ± 2.5 yr; FEV\textsubscript{1}/FVC = 77 ± 2%), and homogenates were analyzed for a wide range of SR properties. Compared with CON, COPD displayed (in μmol·g protein\textsuperscript{-1}·min\textsuperscript{-1}) a 16% lower maximal Ca\textsuperscript{2+}-ATPase activity [maximal velocity (V\textsubscript{max})], 158 ± 10 vs. 133 ± 7, P < 0.05] and a 17% lower Ca\textsuperscript{2+} uptake (4.65 ± 0.039 vs. 3.85 ± 0.26, P < 0.05) that occurred in the absence of differences in Ca\textsuperscript{2+} release. The lower V\textsubscript{max} in COPD was also accompanied by an 11% lower (P < 0.05) Ca\textsuperscript{2+} sensitivity, as measured by the Hill coefficient (defined as the relationship between Ca\textsuperscript{2+} activity and free cytosolic Ca\textsuperscript{2+} concentration for 10–90% V\textsubscript{max}). For the sarco(endo)plasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) isoforms, SERCA1a was 16% higher (P < 0.05) and SERCA2a was 14% lower (P < 0.05) in COPD. It is concluded that moderate to severe COPD results in abnormalities in SR Ca\textsuperscript{2+}-ATPase properties that cannot be explained by changes in the SERCA isoform phenotypes. The reduced catalytic properties of SERCA in COPD suggest a disturbance in Ca\textsuperscript{2+} cycling, possibly resulting in impairment in Ca\textsuperscript{2+}-mediated mechanical function and/or second messenger regulative processes.

skeletal muscle; lung disease; sarcoplasmic reticulum; calcium regulation

There is evidence that intrinsic differences occur in the locomotor muscles of individuals with chronic obstructive pulmonary disease (COPD) when compared with age-matched healthy controls. Most consistently cited have been differences in the potential of the various metabolic pathways and segments. Accordingly, studies have been published indicating a reduced maximal activity of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase (23, 27), representative enzymes of the tricarboxylic acid cycle and fat oxidation (β-oxidation), respectively. Interestingly, the glycolytic potential, as measured by phosphofructokinase and lactate dehydrogenase (LDH), appears either unaltered (41) or increased (23) by the disease. It should be noted that the remodeling of the metabolic pathways that occurs in COPD appears to depend on the location and function of the muscle examined (16, 39, 40). Because mitochondrial potential is sensitive to the history of contractile activity (34), the lower enzyme activities in COPD are frequently attributed to reduced levels of physical activity in this group (18).

Other intrinsic differences also appear to exist in the locomotor muscles of patients with COPD. Reports have been published describing a higher proportion of type II (fast-twitch) and a lower proportion of type I (slow-twitch) fibers in COPD, using both histochemical (51) and electrophoretic-based measurements (17, 28). The fiber-type phenotype is based on the myosin composition of the cell and, in particular, the heavy-chain isoform composition (43).

Surprisingly, little attention has been given to the status of the excitation-contraction coupling processes and the regulation of free cytosolic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}). Precise regulation of [Ca\textsuperscript{2+}]\textsubscript{i} is vital for skeletal muscle function and differentiation given the role of [Ca\textsuperscript{2+}]\textsubscript{i} in activation of the myofibrillar complex and as a signaling messenger involved in multiple processes (3). Ca\textsuperscript{2+} cycling in the muscle cell that regulates [Ca\textsuperscript{2+}]\textsubscript{i} is mediated by the sarcoplasmic reticulum (SR), an organelle within the cell that functions to store, release, and sequester Ca\textsuperscript{2+} (29). The Ca\textsuperscript{2+}-release function is regulated by the ryanodine receptor (RyR), a specialized protein concentrated mainly in the junctional cisternae (functional face) region that acts as a Ca\textsuperscript{2+} channel (15). In human skeletal muscle, regardless of fiber type composition, RyR exists only as a single isoform, namely RyR1 (15). Ca\textsuperscript{2+} uptake occurs in the longitudinal tubules and is controlled by the sarco(endo)plasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA), an enzyme complex that when activated uses the energy generated by the hydrolysis of ATP to sequester Ca\textsuperscript{2+} in the SR (29). In contrast to RyR, SERCA exists as 1a and 2a isoforms in skeletal muscle that are primarily distributed in type II and type I fibers, respectively (53). Type II as contrasted to type I fibers also contain a greater concentration of both RyR and SERCA proteins, which appear to be the major factors accounting for the much more rapid Ca\textsuperscript{2+}-release and Ca\textsuperscript{2+}-uptake observed in the fast-twitch fibers (29).

Given the apparent shift that occurs in COPD toward a greater proportion of type II fibers in the vastus lateralis, it might be expected that the SR protein would also be more highly expressed, resulting in higher activities of the Ca\textsuperscript{2+}-
ATPase, a greater abundance of SERCA1a, and greater rates of Ca\(^{2+}\) uptake and Ca\(^{2+}\) release. Because there does not appear to be any differences between the SERCA isoforms in Ca\(^{2+}\) sensitivity (24), these shifts should occur in the absence of such changes.

There are also other reasons why differences may exist in the properties of the SR between COPD patients and healthy controls. Selected proteins of the SR and, in particular, SERCA, are known to be susceptible to damage inflicted by the accumulation of reactive oxygen species (ROS) (3, 21, 50). Recent evidence suggests that the oxidation and nitrosylation occurring to a range of proteins in COPD are due to the increase of ROS (2). Moreover, the aging process itself has been shown to result in ROS damage to SERCA in rat skeletal muscle and specifically to SERCA2a, the predominant isoform in type I fibers (49). Given the advanced age of patients displaying COPD, it is possible that both the disease and the age might interact to reduce SERCA1a content, possibly resulting in a depression of SERCA activity. However, at present, little evidence exists to support either the ROS-mediated hypothesis or the disease-age interaction. Consequently, at present, suggestions of changes in SR properties accompanying COPD should be based more realistically on the fiber-type transformations that occur.

The purpose of this study was to investigate the differences in the properties of the SR in vastus lateralis muscle between COPD patients and age-matched healthy controls. We have hypothesized, based on the higher proportion of type II fibers, that the COPD patients would exhibit higher \(V_{\text{max}}\) in the absence of changes in Ca\(^{2+}\) sensitivity and higher Ca\(^{2+}\) uptake and Ca\(^{2+}\) release rates when assessed in vitro in homogenates. These differences would be accompanied by lower concentrations of SERCA2a and higher concentrations of SERCA1a.

**METHODS**

**Subjects.** Patients with a diagnosis of moderate to severe COPD (5) were included if they satisfied the following criteria: 1) forced expiratory volume in 1 s (FEV\(_1\)) <60% predicted and forced expiratory volume in 1 s/forced vital capacity (FEV\(_1\)/FVC) <0.7; 2) age ≥40 yr; 3) clinically stable with no exacerbations or change of respiratory medications in the preceding 4 wk; 4) an absence of other unstable medical conditions, i.e., metabolic, cardiovascular, or other respiratory diseases; and 5) a resting arterial oxygen tension (Pa\(_{\text{O}}\)) ≤60 mmHg and an oxygen saturation >75% during pulmonary function testing and cycle exercise testing on room air. A group of healthy, age-matched control subjects was included if they had normal spirometry (FEV\(_1\) ≥80% predicted, FEV\(_1\)/FVC ≥0.7) and an absence of significant health problems, including cardiovascular, neuromuscular, musculoskeletal, or respiratory diseases. Subjects with an allergy to the local anesthetic, lidocaine, were excluded from the study. Patients with COPD were recruited from outpatient respirology clinics and from a database of patients who had completed previous research studies; healthy subjects were recruited from the local community.

**Study design.** This was a controlled, cross-sectional study in which informed consent was obtained from all subjects, and ethical approval was received from the University and Hospital Health Sciences Human Research Ethics Board. After initial medical screening, each subject completed pulmonary function tests (spirometry, body plethysmography, single-breath diffusing capacity), anthropometric measurements, venous blood sampling (routine chemistry and hematology, clotting factors), blood gas sampling, and a symptom-limited cardiopulmonary cycle exercise test. In a subsequent visit, a muscle biopsy was taken from the vastus lateralis muscle with the subject in the supine position. A follow-up visit was conducted ~1 wk after tissue sampling to ensure satisfactory healing of the sample site.

**Tissue sampling.** For tissue sampling, the subjects were required to visit the laboratory on one occasion. During this visit, tissue samples were obtained from the vastus lateralis muscle of the dominant leg using the needle biopsy technique (4), by a trained research associate with extensive experience in this procedure. Care was taken to standardize the region and the depth from which the tissue was sampled. Further details regarding the preparation of the site, the location, and technique used appear in previous publications from our laboratory (10). Two separate tissue samples were extracted using suction to increase yield. All participants, regardless of group, were requested not to eat or to consume caffeinated beverages for at least 4 h before their scheduled visit. Tissue sampling was performed during the morning and early afternoon.

**Analytical techniques.** The tissue sample was used for the measurement of a range of SR-dependent Ca\(^{2+}\)-cycling properties. The properties included the catalytic behavior of SERCA, which consisted of the maximal activity (\(V_{\text{max}}\)), the \([\text{Ca}^{2+}]_i\) needed to elicit 50% \(V_{\text{max}}\) (Ca\(_{1/2}\)), and the slope of the relation (\(E_{\text{Hill}}\)) of [Ca\(^{2+}\)] (Hill coefficient, \(n_H\)). These properties were assessed on homogenates that had been prepared immediately following extraction of the tissue and then stored at −80°C, pending analyses. We have previously shown that \(V_{\text{max}}\) is reduced by ~15–20% if the homogenate is not prepared before freezing (6). The stored homogenate was also used for the measurement of both Ca\(^{2+}\) uptake and Ca\(^{2+}\) release. The distribution of the two SERCA isoforms, known to be expressed in human skeletal muscle, namely SERCA1a and SERCA2a, was measured on tissue that had been frozen and stored under conditions similar to the homogenates.

For the measurement of the catalytic properties of the Ca\(^{2+}\)-ATPase, Ca\(^{2+}\) uptake, and Ca\(^{2+}\) release, homogenates were prepared using ~20–30 mg tissue diluted in ice-cold buffer (1:11 wt/vol) containing (in mM) 250 sucrose, 5 HEPES, 10 NaN\(_3\), and 0.2 phenylmethylsulfonyl fluoride (PMSF) (pH 7.5) by using a handheld glass homogenizer (Duall 20; Kontes). For the Ca\(^{2+}\)-ATPase, the reaction mixture (pH 7.0) contained 200 mM KCl, 20 mM HEPES, 15 mM MgCl\(_2\), 1 mM EGTA, 10 mM NaN\(_3\), 5 mM ATP, 0.3 mM NADH, 10 mM phosphoenolpyruvate (PEP), 18 U/ml LDH, 18 U/ml pyruvate kinase (PK), and 25 μM homogenate. Enzyme activity was measured using a spectrophotometric method (45), as modified by our laboratory (9). All samples were measured in duplicate at 37°C. To measure total Ca\(^{2+}\)-dependent ATPase activity, serial additions of 0.5 μl of 100 mM CaCl\(_2\) were added and continued until a plateau and subsequent decline in Ca\(^{2+}\)-ATPase activity were observed. The Ca\(^{2+}\)-dependent ATPase (Ca\(^{2+}\)-ATPase) activity was calculated as the difference between the total Ca\(^{2+}\)-ATPase activity and the basal or Ca\(^{2+}\)-independent activity. Basal or Ca\(^{2+}\)-independent activity was assessed using 40 μM of cyclopiazonic acid, which completely inhibits the Ca\(^{2+}\)-dependent ATPase (44). To obtain an indirect measure of the integrity of the SR membrane for Ca\(^{2+}\), the Ca\(^{2+}\)-ATPase activity was measured with (1 μM) and without the Ca\(^{2+}\) ionophore A-23187 (C-7522; Sigma). The Ca\(^{2+}\) ionophore prevents the intraluminal accumulation of Ca\(^{2+}\) that is inhibitory to the catalytic activity of the enzyme (45). The ionophore ratio, calculated as the ratio of \(V_{\text{max}}\) with and without the ionophore, is an indirect measure of the degree to which the \(V_{\text{max}}\) is altered as a result of changes in the permeability of the membrane for Ca\(^{2+}\).

In addition to \(V_{\text{max}}\), we calculated Ca\(_{50}\) and the slope of the relationship between [Ca\(^{2+}\)]\(_i\) and Ca\(^{2+}\)-ATPase activity (\(n_H\)), both measures of the sensitivity of the enzyme to [Ca\(^{2+}\)]\(_i\). The Ca\(_{50}\) was obtained from the sigmoid fit of the data while the \(n_H\) was determined through nonlinear regression, using a portion of the curve that corresponded to between 10 and 90% \(V_{\text{max}}\). The concentration of [Ca\(^{2+}\)]\(_i\) used for the calculation of these properties was determined with dual-wavelength spectrofluorometry and the Ca\(^{2+}\) fluorescent dye indo 1. Additional details, including the wavelengths employed, the
dissociation constant, and specific procedures occur in earlier publications from our laboratory (9).

The procedures used to determine Ca\(^{2+}\) uptake and Ca\(^{2+}\) release have also been detailed previously (9) and will only be summarized here. Both properties were measured at 37°C in homogenates using a common assay prepared at the time of the tissue sampling and stored at -80°C. For these measurements, the assay medium consisted of (in mM) 200 KCl, 20 HEPES, 15 MgCl\(_2\), 1.0 mM NaCl, 0.005 EGTA, 5 mM ATP were added to the 2 ml of mixture to initiate the reaction. Ca\(^{2+}\) uptake was measured at four [Ca\(^{2+}\)]\(_i\) of 3.5 μM (with Ca\(_2\)), 5 mM ATP were added to the 2 ml of mixture to initiate the reaction. For Ca\(^{2+}\) release, which was measured as part of the same assay, 20 μM of the releasing agent 4-chloro-m-cresol, a highly specific Ca\(^{2+}\) release agent (22), was added. In our hands, this protocol produces Ca\(^{2+}\)-release kinetics that appear as two phases, an initial fast phase (phase 1) and a slower more delayed phase (phase 2) (47). As with Ca\(^{2+}\)-uptake, phase 1 and phase 2 of Ca\(^{2+}\) release were quantified by differentiating a linear fit curve.

We calculated the ratio between the Ca\(^{2+}\) uptake (2,000 nM) and the V\(_{\max}\) and called this the apparent coupling ratio (12). Because the limited sensitivity of indol 1 for Ca\(^{2+}\) made it uncertain whether we have measured maximal Ca\(^{2+}\) uptake, a condition necessary to employ the term coupling ratio, a revised term was selected. The coupling ratio is a measure of the efficiency of the Ca\(^{2+}\) pump for sequestering Ca\(^{2+}\) in the lumen of the SR (25).

For each sample, all properties were measured in duplicate, and care was taken during each analytical session to match the COPD samples with control samples. Protein was measured in duplicate using the Lowry technique as modified by Schacterle and Pollock (42).

For the SERCA isoform (SERCA1a and SERCA2a) distribution, we used electrophoresis and Western blotting techniques as described in our earlier publications (8, 13). The supernatant of postnuclear homogenates was obtained by centrifugation (10,000 g) for 15 min of a mixture containing frozen tissue and a buffer consisting of 5 mM HEPES (pH 7.5), 250 mM sucrose, 0.2% NaN\(_3\), and 0.1 mM PMSF in a ratio of 15:1 (vol/wt) and then extracted by adding an equal volume of buffer containing 10 mM sodium phosphate (pH 7.4), 150 NaCl, 2% Triton X-100, 2% deoxycholate, 0.2% SDS, 0.2 mM PMSF, and 1,000 IU aprotinin. Protein (10 μg) was used for electrophoresis, which was obtained from the stored (−80°C) postnuclear homogenate. The postnuclear homogenate has been reported to contain 95–99% of the SERCA protein (52).

Electrophoresis of the sample protein was performed with 7.5% SDS-polyacrylamide gels (Bio-Rad Mini-Protein II), equilibrated with transfer buffer, and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad) by placing the gels in transfer buffer and applying voltage (23 V) for 40 min (Trans-Blot Cell; Bio-Rad). The PVDF membranes were blocked for nonspecific binding (10% skim milk powder in Tris-buffered saline, pH 7.5) applied overnight at room temperature. The primary monoclonal antibodies SERCA1a and SERCA2a (A52 and 7E6, respectively, obtained from Affinity Bioreagents) were incubated using dilutions of 1:2,500 (SERCA1a) and 1:1,000 (SERCA2a). After being washed, the PVDF membranes were incubated with a secondary antibody (anti-mouse IgG\(_1\), conjugated to horseradish peroxidase), and the protein was determined by densitometry and an enhanced chemiluminescence immunodetection procedure (Amersham ECL-RPN2106 P1) and visualized using the Chemi Genius imaging system and Gene Snap software (SynGene). Densitometry measurements were performed using Gene Tools software (Syngene). Care was taken to ensure that the signal intensity of the blots was linear over the range of sample protein used.

During a given analytical session, samples for COPD and control were carefully matched, and each sample was run in duplicate with a standard. The average value of the duplicate measurements was initially expressed as a percentage of the standard, and then the COPD samples were expressed as a percentage of the controls, which was set to 100%. The standard used to control for protein loading was based on a sample of tissue obtained from the vastus lateralis in which the postnuclear homogenate was divided into multiple aliquots (stored at −80°C) and a sample used for each analytical session. Protein was also determined measured in duplicate by the technique previously cited (42).

Statistics. The data were analyzed using both ANOVA procedures and Student’s t-tests. For the demographic data, the pulmonary function, and the blood gas data, significant differences between groups were investigated using the Student’s t-test. Student’s t-tests were also employed for the SERCA properties, including the isoform distribution, the apparent coupling ratio, the ionophore ratio, and the Ca\(^{2+}\) release data. A two-way ANOVA with one between-group comparison (control vs. COPD) and one repeated measure ([Ca\(^{2+}\)]\(_i\)) was used to examine for differences in Ca\(^{2+}\) uptake. Statistical significance was accepted at P < 0.05. Throughout the text, statistical differences between means are indicated only as “differences” based on the level of probability established. Data are represented as means ± SE.

RESULTS

Subjects. Eight patients with moderate to severe COPD (6 females and 2 males) and 10 age-matched healthy controls (8 females and 2 males) completed the study. There were no significant differences in age (65.6 ± 3.2 vs. 67.5 ± 2.4 yr), height (159 ± 3 vs. 163 ± 3 cm), weight (73.1 ± 5.3 vs. 74.1 ± 5.5 kg), or body mass index (28.8 ± 1.9 vs. 27.9 ± 1.7 kg/m\(^2\)) between the COPD and control groups, respectively. All COPD patients were in a stable medical regime for at least 3 mo before entry in the study: all used short-acting bronchodilators (β\(_2\)-agonists and anticholinergics, alone or in combination), five used a long-acting β\(_2\)-agonist, and five used inhaled corticosteroids (either alone or in combination with the long-acting β\(_2\)-agonist). All but one of the COPD patients used supplemental oxygen (5 continuous, 1 continuous day time, 1 ambulatory); the single patient who was not using oxygen experienced significant oxygen desaturation during exertion and was actively seeking approval to receive ambulatory oxygen. Two subjects in the control group were taking low doses of short-acting β\(_2\)-agonists and inhaled corticosteroids for a past history of “bronchitis,” but both had normal lung function, one had never smoked, and the other was an ex-smoker that had quit 20 years before the study. At the time of the study, no subjects in either group had used systemic corticosteroids for at least 2 mo. Blood chemistry, hematology, and clotting factors were normal in both groups.

Pulmonary function and blood gas properties. Pulmonary function tests indicated that the COPD patients had significant expiratory airway obstruction (lower FEV\(_1\), FEV\(_1/FVC\), severe hyperinflation (greater total lung capacity, residual volume), and a reduced diffusing capacity compared with the healthy control subjects (Table 1). Arterial blood gases were also compromised in the COPD group as indicated by the lower PaO\(_2\), and higher arterial CO\(_2\) tension compared with controls (Table 1). Compared with healthy controls, COPD patients had a lower oxygen saturation at rest and experienced significant desaturation during exercise (Table 1).

SERCA catalytic properties and isoform distribution. The V\(_{\max}\) in COPD was depressed by ~16% compared with the age-matched healthy controls (Table 2). In addition, the Ca\(^{2+}\) sensitivity of the enzyme, as assessed by both Ca\(_{50}\) and n\\(_H\) was also different between groups. In the case of Ca\(_{50}\), the value was higher in COPD while in the case of n\\(_H\) the value was also

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lower in COPD. Both of these properties are consistent with a reduced Ca\(^{2+}\) sensitivity.

An individual plot of the relationship between SERCA activity and Ca\(^{2+}\) concentration (nM) is provided in Fig. 1 to illustrate the typical relationship that exists between these variables. Differences in both SERCA1a and SERCA2a isoform distribution were also observed between groups (Fig. 2). SERCA1a was observed to be \(\sim 16\%\) higher while SERCA2a was observed to be \(\sim 14\%\) lower in COPD compared with controls, respectively.

The ionophore ratio, an indirect measure of the permeability of the SR membrane to Ca\(^{2+}\), was not different between the groups (3.56 \pm 0.40 vs. 3.85 \pm 0.26).

Ca\(^{2+}\) uptake. The COPD group also displayed an \(\sim 16\%\) lower peak rate of Ca\(^{2+}\) uptake compared with control (4.65 \pm 0.39 vs. 3.85 \pm 0.26 \mu mol g protein\(^{-1}\) min\(^{-1}\)). The lower peak rate of Ca\(^{2+}\) uptake in COPD was also observed at the other submaximal Ca\(^{2+}\) concentrations investigated (Fig. 3). The apparent coupling ratio, which is a measure of peak Ca\(^{2+}\) uptake rate to SERCA \(V_{\text{max}}\), was 0.30 \pm 0.003 for control and 0.029 \pm 0.002 for COPD, a difference that was not significant between groups.

Ca\(^{2+}\) release. In contrast to Ca\(^{2+}\) uptake, Ca\(^{2+}\) release, both phase 1 and phase 2, showed no differences between the COPD and control groups (Fig. 4).

Table 2. Comparison of SERCA kinetic properties in vastus lateralis muscle between chronic obstructive pulmonary disease and healthy control groups

<table>
<thead>
<tr>
<th>CON</th>
<th>COPD</th>
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<tbody>
<tr>
<td>(V_{\text{max}}), \mu mol g protein(^{-1}) min(^{-1})</td>
<td>158 \pm 10</td>
</tr>
<tr>
<td>(C_{50}) (nM)</td>
<td>870 \pm 64</td>
</tr>
<tr>
<td>(n_H)</td>
<td>1.87 \pm 0.06</td>
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Values are means \pm SE. For CON, \(n = 10\); for COPD, \(n = 8\). \(V_{\text{max}}\), maximal Ca\(^{2+}\)-ATPase activity; \(C_{50}\), Ca\(^{2+}\) concentration needed to elicit 50% maximal Ca\(^{2+}\)-ATPase activity; \(n_H\), Hill coefficient determined from Hill plots by using the relationship between free Ca\(^{2+}\) concentration and Ca\(^{2+}\) activity and the section of the curve that corresponds to 10\%–90\% of maximal Ca\(^{2+}\)-ATPase activity. *Significantly different from CON (\(P < 0.05\)).
respectively. It is known that the type II fibers compared with type II fibers and the decreased proportion of type I fibers, are consistent with a shift toward an increased proportion of increase in SERCA1a and the decrease in SERCA2a in COPD I fibers cannot fully explain our results. In this study, only the shift toward increased type II fibers and decreased type fibers cannot fully explain our results. In this study, only the shift toward increased type II fibers and decreased type I fibers (unpublished observations). Based on the differences that we report between the COPD and control groups in the SR properties in this study, it appears that the shift toward increased type II fibers and decreased type I fibers cannot fully explain our results. In this study, only the increase in SERCA1a and the decrease in SERCA2a in COPD are consistent with a shift toward an increased proportion of type II fibers and the decreased proportion of type I fibers, respectively. It is known that the type II fibers compared with type I fibers possess a greater rate of Ca$^{2+}$ uptake, a greater $V_{\text{max}}$ activity, and a greater SERCA1a isoform concentration (36). The lower $V_{\text{max}}$ and Ca$^{2+}$ uptake observed in COPD would appear to be modulated by mechanism(s) associated with the disease state.

One possibility to explain the lower $V_{\text{max}}$ and consequently the lower Ca$^{2+}$ uptake is the arterial hypoxemia that occurs in COPD. It is clear from the pulmonary disturbances displayed by the COPD patients in this study that the disease is moderate to severe, based on the FEV1/FVC and PaO2 reported (33). Because we have found reductions in Ca$^{2+}$ uptake and near-significant reductions in $V_{\text{max}}$ ($P = 0.09$) with altitude acclimatization (19), this possibility is tenable.

Differences in the cellular manifestation of the disease may also depend on the skeletal muscle examined and the contractile history. This is most conspicuous when the diaphragm is compared with the vastus lateralis, the locomotor muscle that is the most often selected for study in COPD. As an example, a recent study investigating the costal region of the diaphragm in COPD has reported a decrease in SERCA1a and an increase in SERCA2a (32), just the opposite of what we report in this paper for the vastus lateralis. The differences between these muscles may also provide insight into the role of contractile activity in the altered cellular phenotype that occurs with COPD. It is well known that the diaphragm in COPD is subject to increased work to sustain an adequate level of gas exchange frequency, the air trapping, and the hyperinflation that occurs (2). At least qualitatively, the differences in cellular properties can be explained as a response to this muscle since the direction of change is similar to what has been observed in chronic overload in healthy muscle (34). In the vastus lateralis muscle, the differences between diseased and nondiseased states have been ascribed to the reduced locomotor activity that typically accompanies COPD (18). The disease has been used to explain the lower potential for oxidative phosphorylation, β-oxidation, and glucose phosphorylation observed with COPD, all of which are very sensitive to contractile activity (51).

Our findings with the SR would suggest that factors other than contractile activity are involved. Increased submaximal contractile activity level is known to elicit a shift in isoform distribution, resulting in greater SERCA2a and lower SERCA1a, and decreases in Ca$^{2+}$ uptake, $V_{\text{max}}$, and Ca$^{2+}$ release (20). The decrease in Ca$^{2+}$ uptake and Ca$^{2+}$ release can be explained, in large part, by decreases in the concentration of the SERCA enzyme and the RyR, respectively (35). With reduced levels of locomotor activity as might be expected in COPD,
these differences would be expected to be reversed, resulting in higher Ca\(^{2+}\)-cycling properties compared with control.

The fact that we have found a lower Ca\(^{2+}\) sensitivity of SERCA with the disease is also suggestive that factors other than contractile activity level are involved. To date, no study investigating chronic increases in activity level in humans have reported an effect on Ca\(^{2+}\) sensitivity.

Another potential factor that may be involved to explain the differences in SR properties observed between groups relates to the impact of ROS. Reports are emerging that COPD is accompanied by increases in selective ROS species (18). The accumulation of ROS is known to alter both SERCA and RyR (3, 21, 50). In the case of SERCA, ROS appears to target a region close to the adenine nucleotide site of the enzyme, resulting in oxidation and nitrosylation, and leading to impaired energy transduction and enzyme function (30). Aging itself has also been reported to result in ROS-mediated damage to SERCA, which appears to alter the SERCA2a isoform located in rat muscle with a predominant type I fiber distribution (49). Because our analytical work was performed on homogenates with a mixed fiber type distribution (38), it was not possible to determine if the differences that we have observed between groups were fiber-type specific, which could add to the credibility of the ROS hypothesis. However, our results are consistent with a recent report that found that in COPD patients SERCA2 was lower in those with the lowest body mass index (31). Moreover, the fact that the lower SERCA2 was also tyrosine-nitrated suggests ROS-mediated involvement.

Disease-induced modifications in the catalytic properties of SERCA could also occur as a result of changes in regulatory factors. Phospholamban (PLN) and sarcopladin are two small proteins that can effect changes in SERCA2a and SERCA1a, respectively (26, 46, 48), through phosphorylation-related processes. The signaling cascades can be activated through epinephrine and/or insulin changes, resulting in activation of kinases via Ca\(^{2+}\)-calmodulin and cAMP-dependent protein mechanisms (26). Reports continue to be published indicating that, in diseases such as chronic heart failure, disturbances may occur in one or more of these signaling cascades (18). It has been shown that phosphorylation of PLN, as an example, can result in a lower SERCA Ca\(^{2+}\) sensitivity (26, 48), similar to what we have observed in this study in the COPD group. Our results provide a solid rationale for investigating whether this mechanism can be implicated in the vastus lateralis muscle in COPD. Unfortunately, given the numerous other properties measured in this study, which will be reported in other papers, no tissue remains to perform these assays.

**Perspectives**

An important issue is whether the changes that we have found in SR function in COPD impacts muscle mechanical properties and fatigability. Although there is consensus that maximal voluntary isometric contraction force of the quadriceps is compromised in COPD patients (18), the difference has been reported to be reduced when the results are adjusted to the cross-sectional area of the muscle (7) or to disappear when muscle strips are removed from the vastus lateralis of COPD patients and assessed for mechanical function in vitro (7). Our findings suggest that some differences may occur. Because the twitch relaxation rate measured in vitro is, in part, determined by the rate of Ca\(^{2+}\) uptake (14), the lower rate that we have observed in COPD should result in a reduced relaxation rate. However, differences in twitch relaxation rate measured in vitro between COPD and healthy controls have not been found (7). It is possible that the effect of the alterations in Ca\(^{2+}\) cycling that we have observed would be manifested with different contractile paradigms. It might be expected that the decrease in the Ca\(^{2+}\) sensitivity of the enzyme would necessitate a greater neural impulse frequency to achieve a given [Ca\(^{2+}\)]\(_i\), and consequently a given force level. This possibility is suggested by a general trend for the force-stimulation frequency to be shifted to the right in COPD muscle (7). Given the need for precise and rapid changes in [Ca\(^{2+}\)]\(_i\), during dynamic activity involving varying velocities and power outputs, it is possible that the effects of altered Ca\(^{2+}\) cycling would be most conspicuous in producing contractile dysfunction under these conditions. Repetitive activity is also known to depress Ca\(^{2+}\) release and Ca\(^{2+}\) uptake and to reduce [Ca\(^{2+}\)]\(_i\), producing fatigue (1). Because COPD patients exhibit disturbances in Ca\(^{2+}\) cycling at rest, it is possible that repetitive activity would provoke additional disturbances. Future specifically designed studies would appear vital to address all these possibilities. Given the need to employ human subjects with COPD, and voluntary exercise, in association with the mixed fiber-type composition of the vastus lateralis, such studies will not only be difficult to design but difficult to interpret. It should also be emphasized, given the plethora of regulatory functions documented for [Ca\(^{2+}\)]\(_i\), that the abnormal Ca\(^{2+}\)-cycling responses observed in COPD would be expected to have far-reaching consequences (3). Indeed, based on the energetic costs associated with Ca\(^{2+}\) cycling in muscle (3), the disturbances in Ca\(^{2+}\) cycling that we have observed in vitro may also be a factor in the reduced mechanical efficiency previously reported in COPD (37). It is important to emphasize that the measurements of the SR Ca\(^{2+}\)-cycling properties are performed “in vitro” under optimal conditions (45). Differences in the intracellular environment “in-vivo” mediated by the disease itself, such as changes in temperature, substrate, and metabolic status, would all be expected to exert a specific effect on Ca\(^{2+}\) cycling, resulting in modifications of the response from that observed in vitro.

In summary, numerous factors are known to affect the properties of the SR and Ca\(^{2+}\) cycling in skeletal muscle (3, 11, 12). Differences between patients and healthy controls in physical activity level, nature, and amount of macronutrient intake and the medications used to treat the disease may all confound the isolation of changes due to COPD itself.

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**REFERENCES**


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